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(54) Title: ADJUVANT AND CELL MATURATION AGENT

(57) Abstract

Use of CD38, or a portion thereof or analogue thereof which can inhibit the binding of CD38 to a non–follicular dendritic cell (DC) or a follicular dendritic cell (FDC) and which retains the ability of CD38 to stimulate a DC or FDC, for use in the manufacture of an adjuvant for use in immunotherapy. Also provided is a method of maturing DCs *in vitro* and a vaccine comprising mature DCs. The invention also provides a natural ligand of CD38 on FDCs and DCs and inhibitors of the ligand. The inhibitors can be used for immunosuppression.

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ADJUVANT AND CELL MATURATION AGENT

The invention relates to the use of CD38, or a portion or analogue thereof, as an adjuvant or to cause maturation of dendritic cells *in vitro*; and to uses of the mature dendritic cells. The invention also relates to the natural ligand of CD38 on follicular and non-follicular dendritic cells and to a method of identifying adjuvants.

Follicular dendritic cells (FDCs) and non-follicular dendritic cells (DCs) present antigen to T cells and B cells. FDC networks are present in lymph nodes and play a critical role in the development of memory B cells in germinal centres. DCs are important in the development of primary T cell responses, particularly CD8 T cell responses.

Although the role of CD38 has previously been a matter of much investigation, the effect of CD38 on dendritic cells has not been investigated. The inventors have shown that administration of a CD38 construct increases the size and number of FDC networks in germinal centres. This would cause an increased efficiency in antigen presentation to B cells and result in an improvement in long term B cell memory and recall responses.

The inventors have also shown that the CD38 construct causes maturation of DCs. Since immature DCs are better than mature DCs in taking up antigen and mature DCs are better at stimulating primary T cell responses, this property of CD38 can be used when preparing DCs *in vitro*. Immature DCs can be used to take up antigen and then be treated with CD38 to change the phenotype to the mature form. Such mature DCs will be more efficient at stimulating primary T cell responses (for example *in vitro* or after administration to a patient).

Using the CD38 construct the inventors have also identified the natural ligand of CD38 on FDC and DC.

The invention thus provides use of CD38, or a portion thereof or analogue thereof which can inhibit the binding of CD38 to a non-follicular dendritic cell (DC) or a follicular dendritic cell (FDC) and which retains the ability of CD38 to stimulate a DC or FDC, for use in the manufacture of an adjuvant for use in immunotherapy. Such an adjuvant may be administered together with, or separately from, an antigen for immunisation. It may, for example, preferably be administered several days after

immunisation, e.g. 4-5 days, after immunisation.

The invention also provides a method of causing maturation of DCs comprising contacting DCs *ex vivo* with CD38, or a CD38 portion or analogue of the invention. The invention additionally provides an *ex vivo* mature DC that has been made using the method.

The invention provides (i) the native ligand present on a FDC or DC which binds CD38 and which is substantially free of FDC or DC cell membrane, excluding CD31; (ii) a protein which is at least 70% homologous to (i) and binds CD38; or (iii) a fragment of (i) or (ii) which retains the ability to bind CD38. The invention also provides an inhibitor of the same CD38 ligand which specifically inhibits activation of the ligand by CD38.

The invention is illustrated by the accompanying drawings in which:

Figure 1a shows the molecular weight of the CD38/IgG1 construct in reducing (on the right) and non-reducing (on the left) conditions.

Figure 1b shows the molecular weight of the construct after N-endoglucosidase treatment.

Figure 2 and Figure 3 show cytometry of DCs.

Figure 4 shows CD38 ligand.

Figure 4a shows MHC class I levels on DCs.

Figure 4b shows MHC class II levels on DCs.

Figure 4c shows B7.1 levels on DCs.

Figure 4d shows B7.2 levels on DCs.

Figure 5 shows anti-DNP antibody responses.

Figure 6 shows CTL assay results.

The term 'analogue' as used below also includes portions of CD38.

The CD38 used in the invention is generally a mammalian or avian CD38, such as a human, primate or rodent CD38 (e.g. mouse or rat CD38). Thus the CD38 can be any of the different species forms, or any of the naturally occurring allelic forms (including variants in an individual such as splice variants) which occur naturally in such animals. Generally such forms of CD38 will be able to bind FDCs and/or DCs and will be able to stimulate FDCs and/or DCs.

The CD38 may be one which is soluble *in vivo*, e.g. a naturally occurring soluble form. Such naturally-occurring soluble forms have previously been described (see, for example Funaro *et al.*, Int. Immunol. (1996) 8, 1643-1650 and Horenstein *et al.*, Biochem. J. (1998) 330, 1129-1135.)

When CD38 (or an analogue with CD38 sequence) is administered to humans according to the invention, it is preferably human CD38 (or preferably the analogue has human CD38 sequence). Human CD38 has previously been studied and techniques for obtaining the extracellular domains of human CD38 in soluble form have also previously been described (see, for example, Daeglio *et al.*, J. Immunol. (1996) 156, 727-734).

The analogue of CD38 can inhibit the binding of CD38 to a FDC or DC. Therefore the amount of CD38 which can bind an FDC or DC in the presence of the analogue is decreased. This is because the analogue is able to bind CD38 ligand on the FDC or DC in a specific manner, and therefore competes with the CD38 for binding to CD38 ligand. The inhibition of binding can be determined using known binding assays, such as those discussed below. FDCs or DCs for use in such assays can be obtained by known methods, such as by sorting cells based on their ability to bind CD38.

Other binding characteristics of the analogue are generally also the same as CD38, and thus typically the analogue binds to antibodies specific for CD38 (e.g. specific for an extracellular portion or the ligand binding site of CD38), and thus inhibits binding of the CD38 to such an antibody.

The analogue retains the ability of CD38 to stimulate FDCs and/or DCs. Thus typically the analogue is able to cause an increase in the FDC networks and/or maturation of DCs (the maturation generally determined by measuring the upregulation of cell surface markers, such as MHC class I or II, or B7.2, on the DCs).

In one embodiment the analogue has ADP-ribosyl transferase activity. It typically has at least 10%, for example at least 20, 40, 60, 100, 150, 200% or more of the ADP-ribosyl transferase activity of CD38.

In one embodiment the analogue is a peptide or comprises 1, 2, 3 or more peptides typically joined together by peptide or non-peptide linkers. The analogue

may comprise sequence from two or more of the proteins discussed below either (e.g. fragments from the proteins) as a fusion protein or joined together by the linker(s).

A peptide analogue or a peptide which is present in an analogue typically has or comprises the same sequence as all or part of CD38, or a sequence which is homologous to part of or all of CD38.

The peptide may comprise a fragment of CD38 or a fragment of the homologous sequence, typically with a length of at least 10, 20, 30, 50 or 100 amino acids long. Such a fragment may be the extracellular portion or ligand binding site of CD38, or the equivalent sequence in the homologous sequence. In a preferred embodiment the fragment is the portion of CD38 which is encoded by the polynucleotide sequence which is amplified when SEQ ID NO:1 and 2 are used as primers in a PCR reaction in which a murine CD38 gene is used as template. The portion of human CD38 which is equivalent to such an amplified sequence (the 'equivalent' sequence is determined based on homology, e.g. using the PILEUP program in the UWGCG package) is also preferred and may be obtained in similar manner.

The peptide analogue or peptide which is present in the analogue may comprise sequence from other proteins (typically naturally occurring proteins). The sequence may be from an antibody (e.g. IgG), such as a Fab, $(Fab)_2$ fragment or Fc fragment. Such an antibody may be one which can bind CD38 ligand or a cell surface protein on a FDC or DC. In a preferred embodiment the peptide is a fusion protein which comprises an extracellular domain portion of CD38 and a non-CD38 sequence.

A preferred analogue comprises a fusion protein of the extracellular domain of CD38 (such as the murine fragment discussed above encoded by the sequence amplified by SEQ ID NO:1 and SEQ ID NO:2, or the equivalent human fragment) and the CH2CH3 (Fc) domains of a human IgG1.

The sequence may be one that causes the analogue to associate with FDCs or DCs, such as the cell membranes of these cells or with proteins on the surface of these cells. Such sequence may be from a protein that binds CD38 ligand or that binds a cell surface protein of FDCs or DCs.

Typically the peptide analogue or peptide present in the analogue comprises 1, 2, 3, 4 or more modifications, which may be natural post-translational modifications or artificial modifications. The modifications are typically the same as the modifications (e.g. the glycosylation) present on natural CD38, typically at the same or equivalent positions. The modification may provide a chemical moiety (typically by substitution of a hydrogen, e.g. the hydrogen of a C-H bond in the peptide), such as an amino, acetyl, hydroxy or halogen (e.g. fluorine) group or carbohydrate group. Typically the modification is present on the N or C terminus of the peptide.

The analogue (or the peptide of the analogue) may comprise one or more non-natural amino acids, e.g. amino acids with a side chain different from natural amino acids. Generally, the non-natural amino acid will have an N terminus and/or a C terminus. The non-natural amino acid may be an L-amino acid.

The analogue typically has or comprises a part which has a shape, size, flexibility or electronic configuration which is substantially similar to CD38 or any of the fragments of CD38 discussed above. It is typically a derivative of CD38 or of such a fragment.

The analogue may be soluble or insoluble in water. The analogue may be capable of forming an oil-in-water or water-in-oil emulsion. The analogue may be capable of associating with a lipid membrane, such as a cell membrane.

The analogue is typically designed by computational means and then synthesised using methods known in the art. Alternatively the analogue can be selected from a library of compounds. The library may be a combinatorial library or a display library, such as a phage display library. Analogues are generally selected from the library based on their ability to mimic the binding characteristics and/or the ability to stimulate a FDC or DC. Thus they may be selected based on ability to bind a CD38 ligand (for example on a FDC or DC) or antibody which binds CD38.

<u>Substances that provide CD38 or the analogue *in vivo*</u>

As discussed above CD38 or an analogue can be used as an adjuvant in vivo. In one embodiment a substance capable of providing CD38 or the analogue in vivo

can be administered. Such a substance is included in the term 'analogue' herein.

The substance is typically a precursor of CD38 or the analogue and is capable of being modified (e.g. hydrolysed) in vivo, typically in a cell, to provide CD38 or the analogue.

The substance may be polynucleotide capable of being expressed to provide CD38, the analogue or the precursor. The polynucleotide is typically DNA or RNA, and is single or double stranded. The polynucleotide generally comprises sequence that encodes CD38, the analogue or the precursor. The coding sequence is typically operably linked to a control sequence (e.g. a promoter) capable of providing for expression of the polynucleotide. Thus typically the polynucleotide comprises 5' and 3' to the coding sequence sequences which aid expression, such as transcription and/or translation of the coding sequence.

The polynucleotide is typically capable of being expressed in the cells of any of the animals mentioned herein. The polynucleotide may be present in a viral or cellular vector.

The invention provides CD38 or an analogue for use as an adjuvant, in particular for stimulating a larger and/or longer lasting immune response against an antigen. The immune response may be an antibody response, or a CD4 or CD8 T cell response. CD38 or the analogue are also provided for increasing the length of time of B cell memory; and/or for increasing the size and/or number of FDC networks. In the case of an antibody response CD38 or the analogue are provided to increase the amount of specific antibodies and/or to increase the time for which such antibodies are produced. In the case of CD4 and CD8 cells the CD38 or analogue are provided to increase the numbers of such cells produced which are specific for the antigen.

The invention provides CD38 or an analogue for use in causing maturation of DCs *in vivo*.

Generally in the method of causing maturation of a DC *in vitro* 10^3 to 10^{12} (e.g. 10^6 to 10^9) DCs are present. Typically CD38 or the analogue are contacted with DCs at a concentration of 10^{-2} to 10^3 µg/ml (e.g. 1 to 10 µg/ml). The DCs are typically contacted with CD38 or the analogue for from 1 hour to 7 days (e.g. from 6

hours to 3 days). Generally the DCs will be contacted in conditions which support them (i.e. which keep them alive) and typically allow the DCs to grow or replicate.

In the method the DCs may also be contacted with other agents which bind proteins on the surface of the DCs (e.g. MHC class I or II molecules, or CD40) and/or which stimulate the DCs. Suitable agents include antibodies to the surface proteins or the natural receptors of the surface proteins. Soluble (for example truncated) forms of the receptors may be used or cells may be used which naturally express the receptors on their surface.

The invention provides a vaccine which comprises CD38 or the analogue and an antigenic component. Such a vaccine is generally capable of stimulating an immune response to the antigenic component, such as any of the immune responses discussed herein. Thus the antigenic component will comprise antibody or T cell (e.g. CD4 or CD8) epitopes.

The vaccine may also comprise other adjuvants or delivery systems, such as adjuvants which stimulate a CD8 T cell response. In a preferred embodiment the antigenic component of the vaccine comprises a CD8 epitope.

The antigenic component is typically from a cancer cell (e.g. specific to a cancer cell, such as a neo-antigen) or a pathogen. The cancer or pathogen are typically ones which can be damaged or killed by an antibody or CD8 T cell response. The pathogen may be an intracellular or extracellular pathogen (e.g. a bacterium or virus).

The invention also provides an *ex vivo* mature DC which has been made using the method of the invention. As discussed below such a cell may be in an isolated or purified form. The cell may be in a composition which also comprises T cells or B cells. The cell may be in a composition of mononuclear cells (e.g. from peripheral blood). The mature DC may have been provided with antigen (such as any of the antigens, or proteins comprising any of the antigens, discussed above which are present in the antigenic component) when it was immature. Such a mature DC will generally comprise the antigen inside the cell (e.g. in the class I or II antigen processing pathway) or on its surface bound to MHC molecules.

Thus in one embodiment an immature DC is contacted with any of the

antigens or proteins discussed above (typically under conditions in which the DC is able to take in the antigen and protein and process it) and is then contacted with CD38 or the analogue to provide a mature DC of the invention. Such contacting may preferably be carried out simultaneously with provision of conditions such that the DCs correspond to DCs subject to T cell signalling. Such conditions may be conditions which mimic T cell signalling, e.g. contacting additionally with anti-CD40 and anti-MHC class II as described in the examples. In another embodiment, the antigen is provided inside the DC (in the same manner as discussed above with regards to substances that provide CD38 or the analogue in vivo). Thus the DC may be contacted with a precursor of the antigen or a polynucleotide that is capable of being expressed to provide the antigen.

Mature DCs produced in accordance with the invention typically have higher levels of MHC class I or B7.2 expression than an immature (e.g. splenic) DC, typically at least 2, 4, 6, 10 or more fold higher.

The invention provides a DC of the invention for use in a method of treating the human or animal body by therapy. In particular the DC is provided for use in stimulating a T cell response *in vivo*. Typically the response is a CD8 T cell response. In the case of the DC discussed above which was provided with antigen when immature the immune response is typically directed to the antigen used.

The invention also provides a vaccine comprising a DC of the invention.

The invention provides a method of stimulating T cells specific to an epitope *in vitro* comprising contacting the T cells with a DC of the invention under conditions in which the DC presents the epitope to the T cell. The method may be used to increase the numbers of such T cells. This may be for the purpose of administering them to a patient or to increase their numbers so that they can be detected. The T cells are CD4 or CD8 T cells.

The invention provides the ligand of CD38 as presented by FDC or DC in substantially isolated form (hereinafter referred to as CD38 ligand) and homologues of the ligand; and fragments thereof. Such homologues and fragments are included in the term 'ligand' herein. CD38 ligand may be obtained from a cell membrane (e.g. a FDC or DC cell membrane).

The ligands which are homologues or fragments are capable of binding CD38, and thus can typically inhibit the binding of CD38 to CD38 ligand. Preferred ligands retain the ability to cause CD38 mediated activation of FDCs or DCs when present in the cell membrane of FDCs or DCs.

The ligand is typically substantially free of FDC or DC cell membrane. The ligand may be substantially free of cell membrane or of cellular components. Such cell membranes may be those of prokaryotes or eukaryotes, mammals (such as humans, primates or rodents) or of the animal in which the particular CD38 naturally occurs.

The ligand may be identified (for example in its naturally occurring form) on a gel, such as under non-reducing conditions (see Example 2).

Ligands which are fragments preferably include the extracellular part of the natural CD38 ligand or the CD38 binding site (or the equivalent sequence in a homologue).

The ligand may or may not be able to bind CD38 or an analogue of the invention. The ligand may be soluble in water. The ligand may be able to associate with lipids, such as cell membranes.

The ligand is typically at least 5, 10, 20, 50, 100 or more amino acids in length. The ligand may be present in the form of a fusion protein which has additional amino acid sequence N and/or C terminal to the ligand sequence.

Polynucleotides of the invention also include sequences that encode a ligand of the invention. Such polynucleotides may also be DNA or RNA, and may be single or double stranded.

Polynucleotides of the invention can be incorporated into a recombinant replicable vector. The vector may be used to replicate the nucleic acid in a compatible host cell. Thus a polynucleotide of the invention may be made in a process comprising introducing a polynucleotide of the invention into a replicable vector, introducing the vector into a compatible host cell, and growing the host cell under conditions which bring about replication of the vector. The vector may be recovered from the host cell. Suitable host cells are described below in connection with expression vectors.

In one embodiment the polynucleotide of the invention in a vector is operably linked to a control sequence which is capable of providing for the expression of the coding sequence by the host cell.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

Such vectors may be transformed into a suitable host cell as described above to provide for expression of a ligand of the invention. Thus, in a further aspect the invention provides a process for preparing a ligand of the invention, which process comprises cultivating a host cell transformed or transfected with an expression vector as described above under conditions to provide for expression of the ligand, and recovering the expressed ligand.

The vectors may be for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. Vectors may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell. The vector may also be adapted to be used *in vitro*, for example in a method of gene therapy.

A further embodiment of the invention provides host cells transformed or transfected with the vectors for the replication and expression of polynucleotides of the invention. The cells will be chosen to be compatible with the said vector and may for example be bacterial, yeast, insect or mammalian.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which the expression vector is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe* nmt1 and adh promoters. Mammalian promoters include the metallothionein promoter which can be induced in response to heavy metals such as cadmium. Most

preferably, the expression vectors are possible for use in insect or mammalian cells. For use in insect cells, strong baculovirus promoters such as the polyhedron promoter are preferred. For expression in mammalian cells, strong viral promoters such as the SV40 large T antigen promoter, a CMV promoter or an adenovirus promoter may also be used. All these promoters are readily available in the art.

Suitable cells include cells in which the abovementioned vectors may be expressed. These include microbial cells such as bacteria (e.g. *E. coli)*, mammalian cells such as CHO cells, COS7 cells, P388 cells, HepG2 cells, KB cells, EL4 cells or HeLa cells, insect cells or yeast such as *Saccharomyces*. Baculovirus or vaccinia expression systems may be used.

The invention provides an antibody that binds CD38 ligand and inhibits the binding of CD38 to CD38 ligand. The antibody may be monoclonal or polyclonal. Such antibody may be produced in a process that comprises contacting CD38 with a population of B cells (*in vivo* or *ex vivo*), and then isolating antibody of the invention which is produced by such cells. As discussed below such antibody may be collected from the sera of animals to which CD38 ligand has been administered. Alternatively B cells (e.g. in the form of spleen) may be removed from such an animal, immortalised, and selected based on their ability to produce antibody that binds CD38 ligand. Antibody may be obtained from such selected cells.

An adjuvant may be identified in a process comprising determining whether a candidate substance binds specifically to CD38 ligand, specific binding indicating that the substance is an adjuvant. An adjuvant may be identified in a process comprising determining whether a candidate substance is capable of causing maturation of DCs, a substance which is capable of maturing DCs being an adjuvant. One aspect of the invention provides a method of identifying an adjuvant comprising contacting CD38 ligand with a candidate substance in the presence of CD38 or an analogue and determining whether said substance competes with CD38 or the analogue for binding to CD38 ligand and whether said substance is capable of maturing DCs. Adjuvants identified in these methods can be used in the *in vitro* and *in vivo* methods or vaccine discussed herein in the same manner as CD38 or the analogue.

The invention also provides an inhibitor of CD38 ligand which specifically inhibits activation of CD38 ligand by CD38. Generally such an inhibitor binds CD38 ligand. It may have any of the structural characteristics discussed above in relation to the analogue of CD38. Thus the inhibitor may comprise a peptide with homology to CD38.

The inhibitor can be identified, for example, in a process comprising providing (contacting) a candidate substance to CD38 ligand in conditions in which in the absence of the candidate substance CD38 ligand would be activated, and determining whether the candidate substance causes inhibition of the activation of CD38 ligand.

The invention provides the inhibitor for use in a method of treatment of the human or animal body by therapy. In particular the inhibitor is provided for use in a method of immunotherapy. Such immunotherapy is generally immunosuppression, such as by inhibition of CD38 mediated activity of FDC or DC. Thus the ligand may be used to inhibit the maturation of DC or the activation of T cells by DC *in vivo*. The inhibitor may be used to inhibit the activity of FDC networks *in vivo*, such as by causing a decrease in the number of FDC networks. Thus the inhibitor may be used to decrease FDC mediated B cell activity, which would generally lead to a decreased amount of antibody being produced by the B cells and/or a decrease in the length of time of B cell memory.

Thus the inhibitor is provided for use in a method of treating a disease which is caused by an immune response. The disease may be an autoimmune disease. In one embodiment the disease is one in which the immune response is caused by a foreign agent (such as a pathogen), but the immune response has a deleterious effect on the host.

The inhibitor may also be used to treat DCs or FDCs *in vitro* in a process in which the DC or FDC is contacted with the inhibitor, generally in conditions which support (i.e. keep alive) the DC or FDC.

<u>The substances and cells of the invention</u>

The discussion below includes substances provided by the invention,

substances identified in the methods discussed herein, cells provided by the invention and cells which have been produced in the methods discussed herein.

The CD38, analogues, CD38 ligands, antibodies, polynucleotides (e.g. that encode analogues or ligands) or inhibitors (of CD38 ligand) discussed herein may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 70%, e.g. at least 80, 90, 95, 97 or 99% of the peptide, polynucleotide or dry mass in the preparation. These substances may be substantially free of cells or of cellular components (such as cell membranes).

The DCs of the invention or the T cells produced in the method of stimulating T cells may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 70%, e.g. at least 80, 90, 95, 97 or 99% of the cells or dry mass in the preparation.

Any of the above substances or cells may be in the form of a pharmaceutical composition which comprises the substance or cell and a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline.

CD38, the analogue or CD38 ligand may be in a soluble form or be associated with lipid, for example a lipid membrane, such as a vesicle or cell membrane. Thus these substances may be present on the surface of a cell, such as a cell on which CD38 or CD 38 ligand is or is not naturally expressed. The cell may be a T cell, B cell, macrophage or NK cell which may be intact or lysed. The CD38, analogue or CD38 ligand may be present in the a preparation made from such a cell, such as an extract (e.g. a partially purified extract) from such a cell.

Any of the above substances may be in any of the above forms when present in the vaccines of the invention or when used in the *in vitro* or *in vivo* methods of the invention.

Methods of producing antibodies

The antibodies mentioned herein may be produced by raising antibody in a host animal. Such antibodies will be specific to CD38 or to the products mentioned above which bind antibodies. CD38 or the products are referred to as the

'immunogen' below. Methods of producing monoclonal and polyclonal antibodies are well-known. A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and isolating immunoglobulins from the serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour cells (Kohler and Milstein (1975) *Nature* **256**, 495-497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat or mouse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled, for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

Homologous sequences

Peptides which have a homologous sequence to a given (original) peptide are discussed herein (for example peptide analogues or peptide(s) present in an analogue which are homologous to CD38, or homologues of CD38 ligand). The discussion below describes how such homologues may be related to the original peptide.

The homologous sequence is typically at least 70% homologous to the original peptide, preferably at least 80 or 90% and more preferably at least 95%, 97% or 99% homologous thereto, for example over a region of at least 20, preferably at

least 30, for instance at least 40, 60 or 100 or more contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on the basis of amino acid identity (sometimes referred to as "hard homology").

Homology can be measured using known methods. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings) (Devereux *et al* (1984) *Nucleic Acids Research* **12**, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S. F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci.* USA 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.*

USA 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The homologous sequence typically differs from the original sequence by substitution, insertion or deletion. Generally from 1, 2, 3, 4 or more substitutions, deletions or insertions, for example over a region of at least 10, preferably at least 20, for instance at least 30, 40, 60 or 100 or more contiguous amino acids in the analogue. Thus the homologous sequence may differ from the original sequence by at least 2, 5, 10, 20, 30 or more substitutions, deletions or insertions.

The substitutions are preferably 'conservative'. These are defined according to the following Table. Amino acids in the same block in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar - uncharged	C S T M
		N Q
	Polar - charged	D E
		K R
AROMATIC		H F W Y

A polynucleotide sequence encoding the homologous peptide typically hybridises with a polynucleotide encoding the original peptide. It typically hybridises at a level significantly above background. The signal level generated by the interaction is typically at least 10 fold, preferably at least 100 fold, as intense as 'background' hybridisation. The intensity of interaction may be measured, for example, by radiolabelling the probe, e.g. with ^{32}P. Selective hybridisation is typically achieved using conditions of medium to high stringency (for example

0.03M sodium chloride and 0.003M sodium citrate at from about 50°C to about 60°C).

Binding

The binding between any two substances mentioned herein is typically a specific binding. The binding may be reversible or non-reversible binding.

Determination of binding, for example in the method of identifying an adjuvant, can be done by using any known binding assay. Typically whether or not a first substance binds to a second substance is done by determining whether the second substance is able to inhibit the binding of a substance known to bind the first substance (for example a specific antibody).

Binding may be determined by measuring a characteristic of any of the substances that changes upon binding, such as spectroscopic changes.

Binding between may be determined in a 'band shift' system, in which the retardation of a substance on a gel can be used to detect when it is bound to another substance. The binding may be determined in a competitive binding method.

Administration

The substances and cells of the invention (in particular CD38, analogues, DCs, vaccines, T cells produced in the method of stimulating T cells, adjuvants identified in the method of identifying an adjuvant and inhibitors CD38 ligand of the invention) are referred to as the 'substances' below. The substances may be administered to a human or animal in need of treatment. The condition of the human or animal can thus be improved.

The invention provides the substances for use in a method of treating the human or animal body by therapy. Thus the substances are provided for use in immunotherapy. The substances are provided for use in the manufacture of an adjuvant (or an immunosuppressant in the case of an inhibitor of CD38 ligand) for use in immunotherapy. Thus the invention provides a method of treating a disease comprising administering a substance of the invention.

The substances may be combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition. Suitable carriers and diluents

include isotonic saline solutions, for example phosphate-buffered saline. The composition may be formulated for parenteral, intramuscular, intravenous, subcutaneous, or transdermal administration.

The dose at which the substance of the invention is administered to a patient will depend upon a variety of factors such as the age, weight and general condition of the patient, the condition that is being treated and the particular substance that is being administered. A suitable dose may however be from 0.1 to 100 mg/kg body weight such as 1 to 40 mg/kg body weight.

Substances which are polynucleotides can be administered directly as a naked nucleic acid construct. Uptake of naked nucleic acid constructs by mammalian cells is enhanced by several known transfection techniques for example those including the use of transfection agents. Example of these agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectam™ and transfectam™). Typically, nucleic acid constructs are mixed with the transfection agent to produce a composition.

When the polynucleotide is delivered to cells by a viral vector, the amount of virus administered is in the range of from 10^6 to 10^{10} pfu, preferably from 10^7 to 10^9 pfu, more preferably about 10^8 pfu for adenoviral vectors. When injected, typically 1-2 ml of virus in a pharmaceutically acceptable suitable carrier or diluent is administered. When the polynucleotide of the invention is administered as a naked nucleic acid, the amount of nucleic acid administered is typically in the range of from 1 μg to 10 mg. The polynucleotide may be administered in a cellular vector.

In the case where the substance is a cell (or the substance is a polynucleotide in a cellular vector) then typically 10^3 to 10^{12} cells are administered, such as 10^6 to 10^9 cells.

The following Examples illustrate the invention:

Example 1

Materials and Methods

Construction of soluble mouse CD38 Human IgG1 Fc Chimeric protein

The primers used to amplify the extracellular domain of mouse CD38 were 5'

(AGG CCG CGC TCA CTC CTG GTG GTG GTG TGG (SEQ ID NO:1)) and 3' (TAC TCA CGT ATT AAG TCT ACA CGA TGG GTG CTC (SEQ ID NO:2)). A cDNA library of splenic cells was used as template for PCR amplification. Each primer contained restriction sites that allowed subcloning into a vector that contained the sequence encoding the CH2CH3 (Fc) domains of human IgG1. The resulting plasmid was transfected into J558L cells. Soluble mouse construct (CD38γ1) was purified from culture supernants using Protein G columns.

In vivo administration of soluble CD38γl

Mice were immunized with 50μg of soluble DNP-KLH (Calbiochem-Novobiochem, USA). Groups of 4 mice were injected i.v. with either CD38γl or human IgG (Binding Site, Birmingham, UK) at 100μg/mouse/day for 4 days starting at the day of immunization.

Effect on FDC networks using tissue sections

Spleen and lymph nodes were collected from mice after 14 days and frozen for cryostat sections. Sections were fixed with either 2% paraformaldehyde or cold acetone. The sections were then stained with either FDC-M1 (FDC and tingible body macrophages), PNA (germinal centres), B220, CD3 or M115.4 (Anti-class II). And anti-Rat-Ig labelled with peroxidase. The number of FDC networks /x20 field, stained by FDC-M1, was counted in spleen sections of 4 treated and 4 untreated mice. Between 11 and 15 fields were counted per section/mouse and a t test used to determine statistical significance of differences in mean number of networks per field.

Distribution of the ligand for CD38

Mice were given SRBC and at the time of immunisation and after 1, 2, 5, 7 and 9 days the spleen and lymph nodes were collected. Tissue sections of spleen and lymph node from naïve and mice immunised with CRBC were labelled with the construct and anti-human IgG-Fc-peroxidase.

Sections were also double labelled with alkaline phosphatase labelled class II

antibody or F480. Some sections were treated with an rat anti-mouse Fc antibody known to block Fc binding and then labelled with the construct, but these sections could not be double labelled.

Flow cytometry

Purified DC (Wykes *et al* (1998), J.Immunol., **161**, 1313-1319) were labelled with the construct and biotin labelled anti-human Ig and Streptavidin-FITC.

Isolation of immature splenic DC

Spleens from 8-10 week old C57BL/6 mice were digested in collagenase D and DNAase (Boehringer Mannheim, UK), RBC were lysed and cells incubated with KT3 (anti-CD3), anti-CD4 (TYS.191.1), anti-μ (B cells), 3D6 (marginal zone metalophillic macrophages) and biotinylated anti-Ig1 and anti-IgG2a antibodies (Binding Site Ltd., UK) for 1h at 4°C. Labelled cells were depleted by rosetting with anti-rat and anti-mouse Ig-coated SRBCs and layering over histopaque (Sigma, USA). Rosetting has the added advantage of depleting macrophages via their Fc receptors. Contaminating cells were depleted using MACS system (Miltenyi Biotec, Germany). The final DC-enriched population was examined by flow cytometry and immunocytochemistry and contained >95% DC based on morphology and expression of MHC Class II, CD11c (very weak expression) and CD 40 (very weak expression), with less than 1% T cells, B cells or macrophages. Dendritic cells were also produced using the adherence method described by Steinman and Cohn (1974) J. Exp. Med., **139**, p380, which was known to produce mature DC. These adherent splenic DC were further treated to remove contaminating T, B cells and macrophages.

Immunoprecipitaion and western Blotting

The ligand for CD38 was immunoprecipitated from DC pulsed with ^{35}S-methionine radiolabel for 4 hours. The ligand was then immunoprecipitated and run on SDS-PAGE gels as described in Wykes *et al* (1998) Euro. J. Immunol. 28, 548).

Maturation of DC

Immature DC were treated with either 30μg/ml Human IgG1 (Binding Site Ltd., UK) or CD38γl. Other treatments included anti-CD40 (FGK-45) and anti-Class II (M115.4), or CD38γl, anti-CD40 and anti-Class II, CD38γl and anti-CD40 or LPS. All antibodies were used at 10μg/ml and the LPS at 50g/ml. The cells were cultured for 18 hours and labelled with antibodies to detect Class I (28.8.6S), class II (Cadarlane, Canada) B7.1 and B7.2.

Example 2

Results

Characterisation of the construct

As indicated above, a chimeric protein of murine CD38 and the Fc portion of human IgG1 (CD38γl) was created and purified on protein G columns. The construct occurs predominantly as a dimer but some monomeric protein is seen under non-reducing conditions (Figure 1a). Under reducing conditions, a single band with an apparent molecular weight of 55kD is observed. The protein was shown to be glycosylated using N-endoglucosidase, with a reduction of approximately 5kD (Figure 1b).

Localization of ligand for CD38 in mice using Immunocytochemistry

CD38γl was used to localise the ligand for CD38 in the spleen and lymph node. Human IgG1 was used to detect any binding due to the Fc portion of Ig. Mice were given SRBC i.v. via the tail vein and the spleen and LN collected at day 0, 2, 5, 7, and 9. The sections were treated with the construct or human IgG1 and immuncytochemistry carried out.

While the human IgG bound occasional cells in the red pulp, the CD38γl bound to different cell types. Five to ten percent of single cells in the red pulp of the spleen expressed the ligand and MHC class II. The ligand was also weakly expressed by dendritic cells in the T cell area of the spleen. Due to weak detection of the ligand in tissue sections, flowcytometry of purified DC (Figure 2) was used to

demonstrate the ligand (Figure 3 and 4). The ligand was also found on networks of cells in the secondary follicles by days 3-5 following immunisation. The networks were FDC that co-expressed FDC-M1, a FDC specific marker. Antibody to Fc receptor was used to treat tissue sections prior to binding of the construct to rule out Fc mediated binding. We did not detect any ligand on T cells or B cells in the appropriate areas (flowcytometry did not show any evidence of binding to T cells and B cells either). There was some very weak staining of the vascular endothelium. The absence of any binding of the construct to cells expressing CD38 rules out homotypic adhesion.

There have been studies that suggested that human CD38 mediates adhesion of lymphoid cells to the endothelium via homotypic adhesion and that CD31 was possibly the receptor for CD38. However, in those studies western blotting was used and no direct binding between CD38 and the CD31 was shown.

Characterisation of the ligand for CD38

Fresh DC were lysed and run on a 3-15% gradient gel under non-reducing conditions. A western blot of the gel was then stained with the construct and anti-human Fc-HRP antibody (Figure 4). Bands of about 130kD, 65 and a doublet at 33kD were seen. A weak band of about 50 kD was also seen, especially in fresh immature DC. An irrelevant cell type or control antibody were not included in these experiments. ^{35}S-methionine/cystine labelling also detected these bands.

CD38 has a role in Germinal centre development in vivo

To identify a function for CD38, groups of mice were given soluble DNP-KLH either with human IgG1 or the CD38γl every day for 4 days. The spleens were collected after 14 days and the sections stained with various markers. Staining with antibodies to class II, B220 or CD3 did not show any change to the architecture or distribution of cells in lymphoid tissue. However, treatment of mice with CD38γl had markedly increased the numbers of FDC networks in GC, stained by the antibody FDC-M1 by at least 2.8 fold (t test, p=<0.001). The apparent size of the germinal centres were also several fold larger as seen by PNA and FDC-M1 staining

and could account for the apparent increase in number of GC.

As B cells express CD38, we suggest that when B cells interact with CD38L on FDC in follicles, this interaction stimulates an expansion of the FDC network. CD38L is the first molecule known to modulate FDC function which has implications for germinal centre and antibody memory development. Germinal centres are essential for the development of antibody memory and expansion and enhancement of this environment should in theory increase the numbers of memory B cells that develop. Furthermore, it has also been shown that antibody memory is dependent on FDC retaining unprocessed antigen on their surface via antibodies. Thus a larger FDC network would also improve recall (memory) responses.

<u>CD38γ1 improves antibody memory</u>

As a follow on from the previous observation that CD38g1 improved the size and numbers of germinal centres, we immunised 2 groups of Balb/c mice with 75μg DNP-KLH and 5d later gave one group 75μg CD38γ1 and the remaining mice 75μg human IgG1 as a negative control. After 10 weeks, lymphois cells were purified from the spleen of these mice and transferred to SCID mice of a B6 background, i.v. along with 10μg DNP-KLH. These SCID mice were also given spleen cells from Balb/c KLH primed mice to provide optimal T cell help. The mice were bled after 8 and 14d (data not shown) and tested for IgH^a haplotype anti-DNP responses. Balb/c immunoglobulin is of IgH^a haplotype and the B6 background is IgH^b. Although SCID mice produce very little or no immunoglobulin, detection reagents specific for the IgH^a haplotype confirmed that the immunoglobulin detected was of donor origin. The response after 8 days indicated memory responses since primary IgG responses take longer to develop.

The titres of IgM anti-DNP were low 8d after the transfer of memory cells in all mice. However, 5/5 mice given control antibody produced IgG1 anti-DNP antibody (Figure 5a) but only 1/5 mice produced a low titre IgG2a anti-DNP antibody (Figure 5b). In contrast, following treatment with CD38γ1 under identical conditions, 5/5 mice produced IgG2a and IgG1 anti-DNP antibody (Figure 5a and b). The titres of IgG2a anti-DNP were 3-fold lower than IgG1 anti-DNP but 10-fold

higher than the single mouse that produced IgG2a anti-DNP.

CD38 has a role in DC maturation

To identify a function for CD38 ligand on DCs, fresh *ex vivo* immature DCs were purified (without adherence) and treated with CD38γl alone or in combination with other antibodies. Human IgG1 was used to control for non-specific effects of Fc binding, although this was unlikely since cells expressing Fc receptors were removed using rosetting. LPS was used to show maximal maturation of DCs. Mature splenic DCs prepared by the adherence technique were used to compare maturity of treated cells. LPS treatment of fresh DCs increased expression of class I, class II and B7.2 to levels comparable with mature DCs. However, B7.1 expression was not significantly affected.

Treatment of the DC with human γl showed minimal changes in expression of all markers whereas treatment with anti-Class II and anti-CD40 (to mimic T cell signalling) induced a 28% increase in the numbers of cells expressing higher levels of class I. This treatment did not increase expression of any other marker. However. treatment with CD38γl increased Class I expression modestly and in combination with anti-CD40, or anti-CD40 and Class II, increased class I expression to levels comparable to LPS treatment (Figure 4a). While the percentage of cells expressing "higher" class I levels was lower than mature splenic DC, the mean fluorescence intensity was higher. MHC class II levels on all treated cells were not markedly different from the mature splenic DC (Figure 4b). Moreover, B7.1 levels were reduced by all treatments especially CD38γl in combination with anti-CD40 and class II when compared to human γl and mature DC (Figure 4c). In contrast, the construct combination improved B7.2 expression to levels equal to LPS treatment and better than mature splenic DC (Figure 4d).

The implications for these observations are that these treated DC may have a role in CTL development. Firstly, the phenotype of LPS treated DC was comparable to mature splenic DC. Furthermore. mature splenic DC have been shown to prime CTL *in vivo*, suggesting that LPS- treated DC may also be capable of priming CTL. Since LPS induces IL-12 production by DC driving a TH1 cytokine profile essential

for CTL responses this may indeed be the case. Secondly, class I and B7.2 expression are essential to prime CTL and these were found on LPS and mature DC but not on immature DC which do not prime CTL. Thus, signalling of class II, CD40 and CD38L will mature DC, as determined by their in change in phenotypic expression, to a form known to prime CTL. In fact the higher B7.2 levels on these cells may suggest a pre-disposition to priming CTL.

The clinical implications are two-fold. Firstly, DC are required to prime CTL but a problem exists in that only immature DC will endocytose antigen. However, these DC will not prime CTL. Conversely, mature DC cannot take up antigen but can prime CTL. Therefore, in a clinical application immature DC, which are easier to obtain, could be pulsed with antigen *ex vivo* and then treated to mature them to a form which would prime CTL.

CD38γ1 treated DC improve cell-specific cytotoxicity

Since studies have shown that the expression of class I and B7.2 were essential for the development of cytotoxic T cells, and our CD38γ1 based treatment improved expression of these molecules, we tested these cells in vivo. Fresh DC were pulsed with P815 tumour lysate and treated with either human IgG1 control antibody or CD38γ1/anti-Class II/anti-CD40 antibodies for 20 hours, washed and given to groups of 3 naïve DBA/2 mice. After 1 week, this process was repeated. After another week, the spleens were collected and cultured with irradiated P815 cells. After the third week, cell counts of spleen cells from CD38γ1 treated DC were 3-fold higher than control treated mice. The absolute numbers of CD4, CD8 and B cells were increased (data not shown). The numbers of NK cells were also apparently increased. CTL assays showed that CTL and non-CTL mediated killing was also improved compared to untreated DC (Figure 6).

Claims

1. Use of CD38, or a portion thereof or analogue thereof which can inhibit the binding of CD38 to a non-follicular dendritic cell (DC) or a follicular dendritic cell (FDC) and which retains the ability of CD38 to stimulate a DC or FDC, for use in the manufacture of an adjuvant for use in immunotherapy.

2. Use according to claim 1 wherein the portion is an extracellular domain portion or the analogue is a fusion protein which comprises an extracellular domain portion of CD38 and a non-CD38 sequence.

3. Method of causing maturation of DCs comprising contacting DCs *ex vivo* with CD38 or a portion or analogue as defined in claim 1 or claim 2.

4. A method as claimed in claim 3 wherein said DCs are simultaneously with said contacting under conditions such that the DCs correspond to DCs subject to T cell signalling.

5. A vaccine comprising an antigenic component and an adjuvant component, wherein said adjuvant component comprises CD38 or a portion or analogue as defined in claim 1 or claim 2.

6. A vaccine according to claim 5 in which the antigenic component comprises a CD8 T cell epitope.

7. An *ex vivo* mature DC that has been made using the method of claim 3 or claim 4.

8. A DC according to claim 7 for use in a method of treating the human or animal body by therapy.

9. A DC according to claim 7 for use in stimulating a T cell response *in vivo*.

10. A vaccine comprising a DC according to claim 7.

11. A method of stimulating T cells specific to an epitope *in vitro* comprising contacting the T cells with a DC according to claim 7 under conditions in which the DC presents the epitope to the T cell.

12. (i) The native ligand present on a FDC or DC which binds CD38 and which is substantially free of FDC or DC cell membrane, excluding CD31;

(ii) a protein which is at least 70% homologous to (i) and binds CD38; or

(iii) a fragment of (i) or (ii) which retains the ability to bind CD38.

13. A polynucleotide that encodes (i), (ii) or (iii) as defined in claim 12.

14. An antibody that binds (i), (ii) or (iii) as defined in claim 12 and inhibits the binding of CD38 to (i), (ii) or (iii).

15. A method of identifying an adjuvant comprising contacting the CD38 ligand present on FDCs or DCs, or (ii) or (iii) as defined in claim 12 with a candidate substance in the presence of CD38 or an analogue as defined in claim 1 and determining whether said substance competes with CD38 or the analogue for binding to the CD38 ligand, (ii) or (iii) and whether said substance is capable of maturing DCs.

16. A use, method or vaccine according to any one of claims 1 to 6 in which the analogue is an adjuvant identified in the method of claim 15.

17. An inhibitor of CD38 ligand as defined in claim 12, which specifically inhibits activation of said ligand by CD38.

18. An inhibitor according to claim 16 for use in immunosuppression.

Fig.1a.

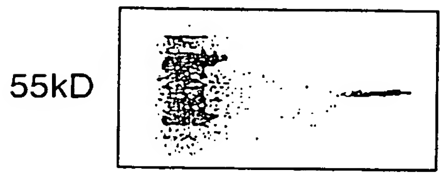


Fig.1b.

Fig.3.

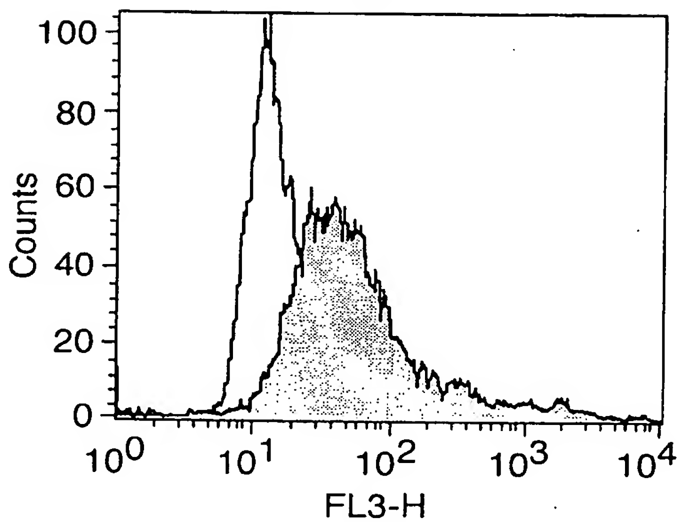


Fig.4.

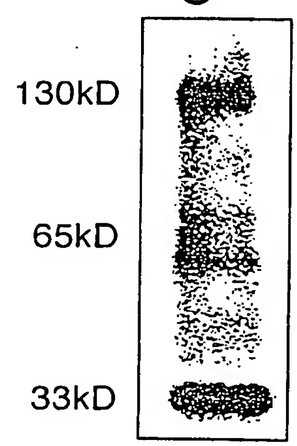


Fig.2.

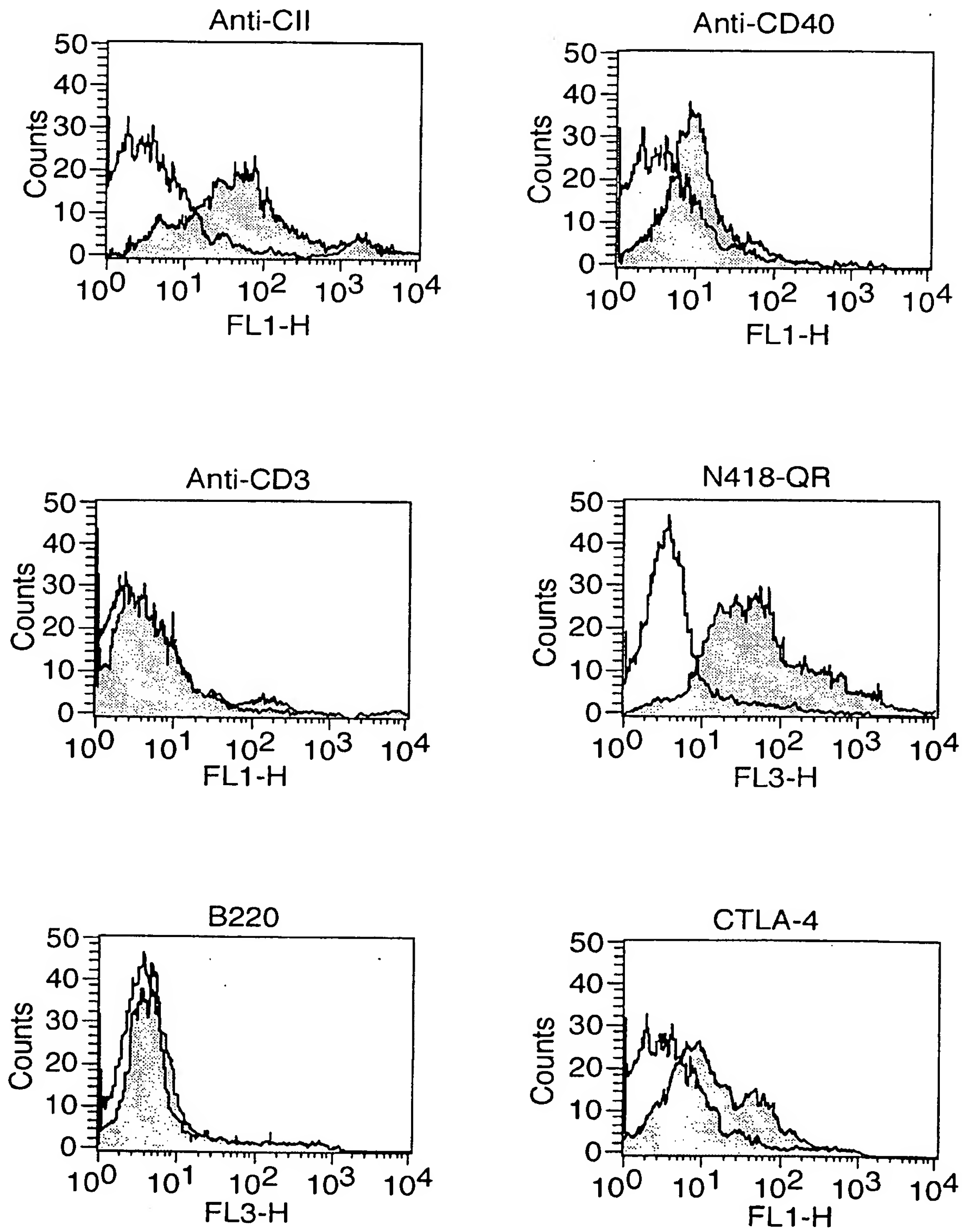

Anti-CII
Counts
FL1-H
Anti-CD40
Counts
FL1-H
Anti-CD3
Counts
FL1-H
N418-QR
Counts
FL3-H
B220
Counts
FL3-H
CTLA-4
Counts
FL1-H

Fig.4a.

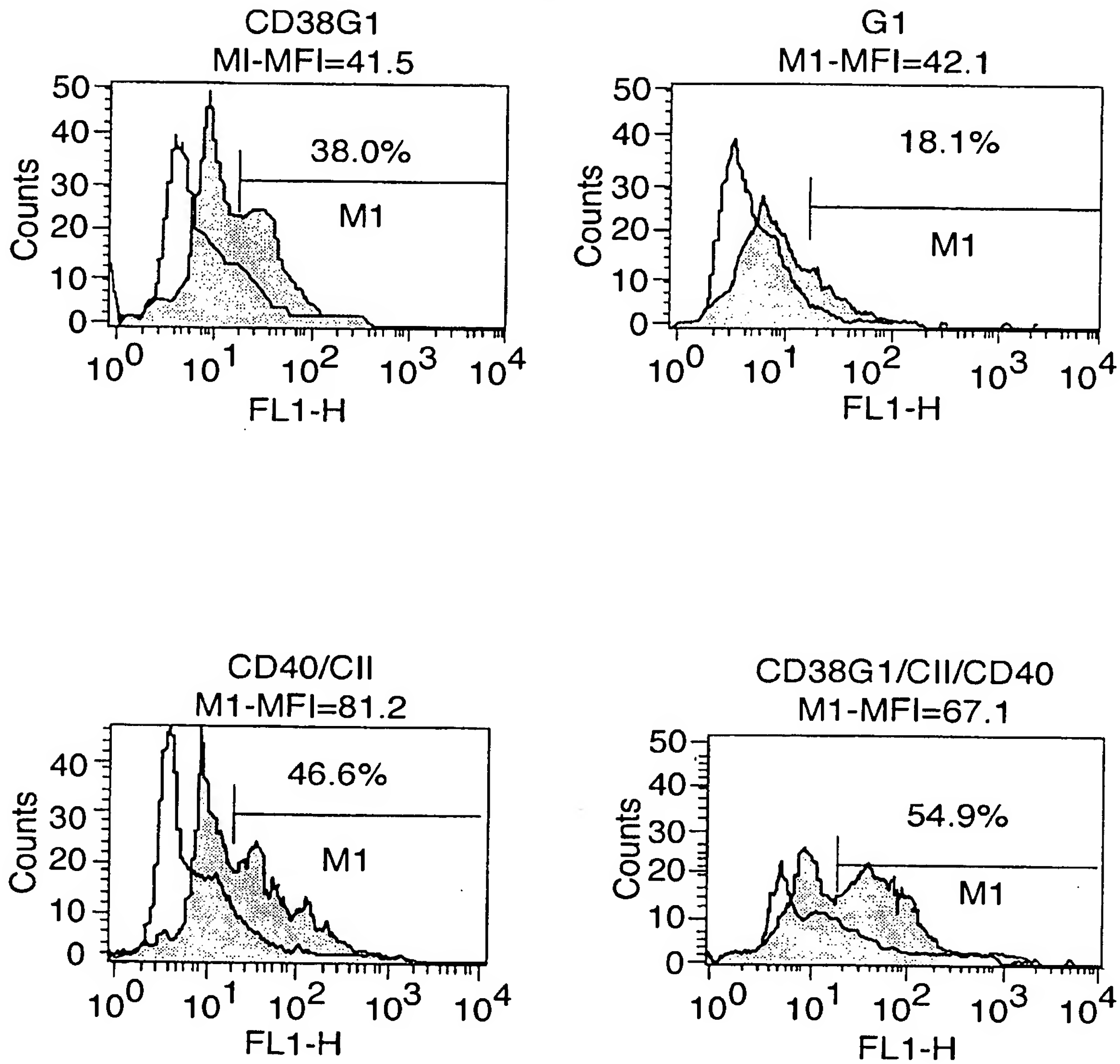
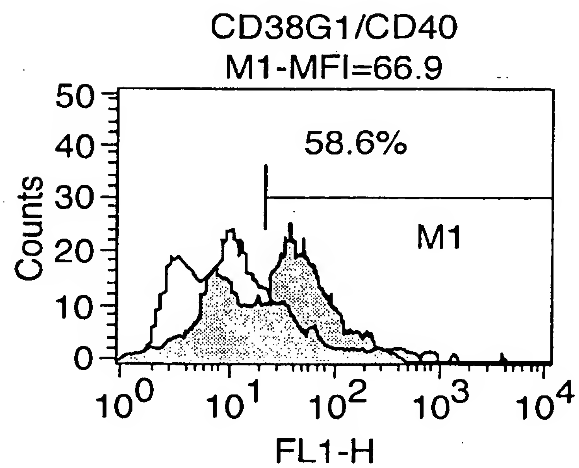
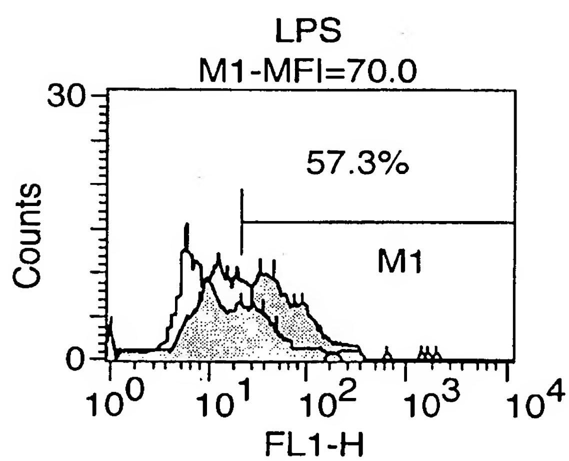

CD38G1
MI-MFI=41.5
38.0%
M1
Counts
FL1-H
G1
M1-MFI=42.1
18.1%
M1
Counts
FL1-H
CD40/CII
M1-MFI=81.2
46.6%
M1
Counts
FL1-H
CD38G1/CII/CD40
M1-MFI=67.1
54.9%
M1
Counts
FL1-H

Fig.4a (Cont).


CD38G1/CD40
M1-MFI=66.9
50
40
30
20
10
0
Counts
58.6%
M1
10^0 10^1 10^2 10^3 10^4
FL1-H


LPS
M1-MFI=70.0
30
0
Counts
57.3%
M1
10^0 10^1 10^2 10^3 10^4
FL1-H

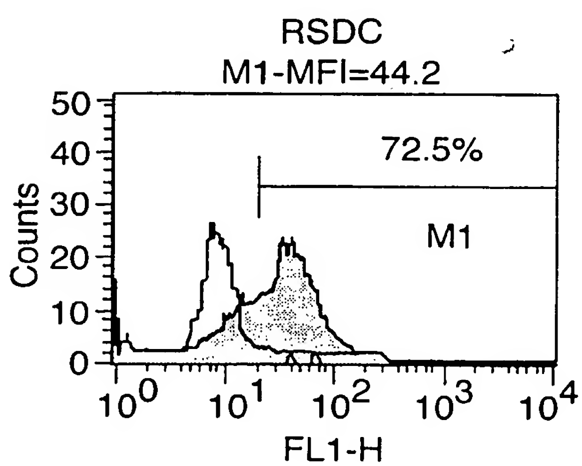

RSDC
M1-MFI=44.2
50
40
30
20
10
0
Counts
72.5%
M1
10^0 10^1 10^2 10^3 10^4
FL1-H

Fig.4b.

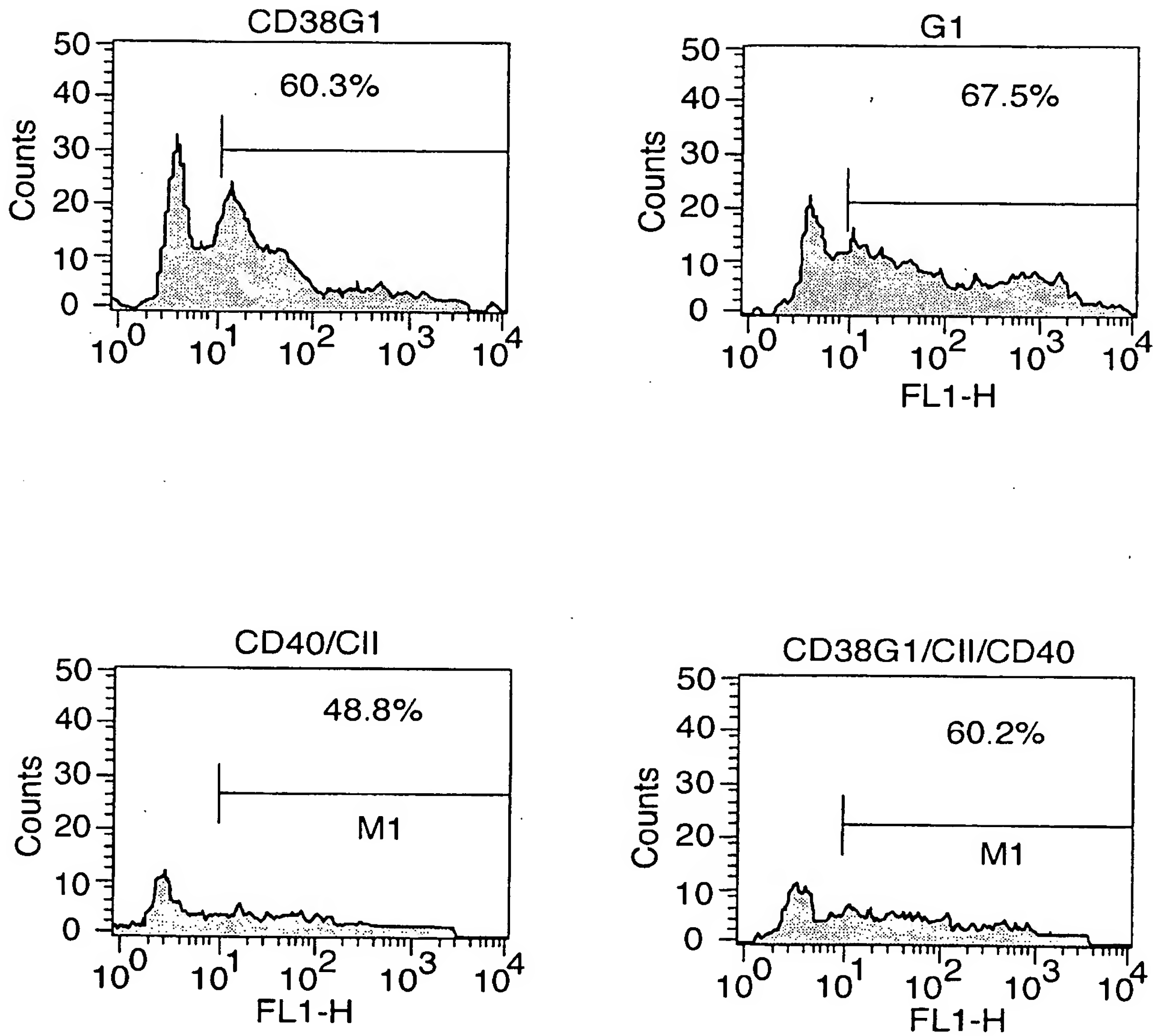
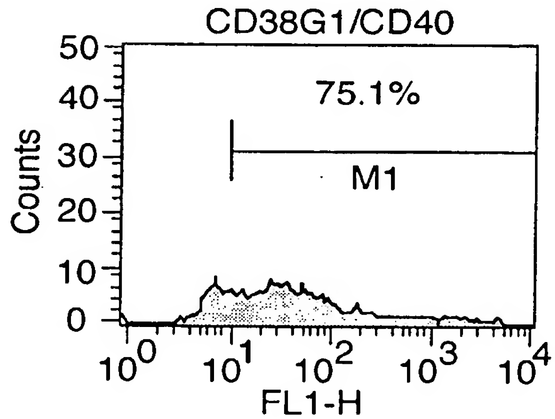
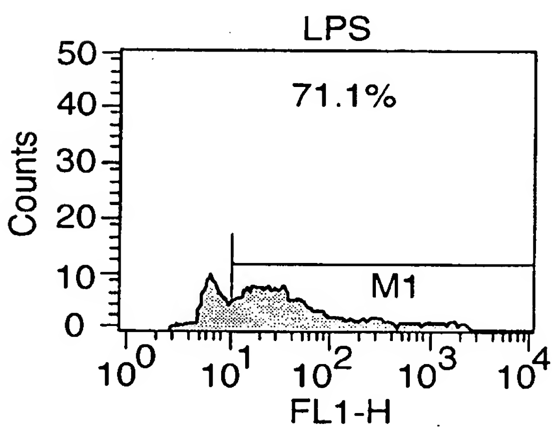

CD38G1
60.3%
Counts
50
40
30
20
10
0
10^0 10^1 10^2 10^3 10^4
G1
67.5%
Counts
FL1-H
CD40/CII
48.8%
M1
Counts
FL1-H
CD38G1/CII/CD40
60.2%
M1
Counts
FL1-H

Fig.4b(Cont).


CD38G1/CD40
75.1%
M1
Counts
50
40
30
20
10
0
10^0 10^1 10^2 10^3 10^4
FL1-H


LPS
71.1%
M1
Counts
50
40
30
20
10
0
10^0 10^1 10^2 10^3 10^4
FL1-H

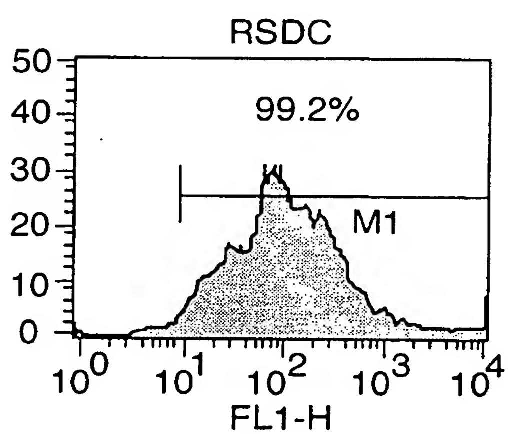

RSDC
99.2%
M1
50
40
30
20
10
0
10^0 10^1 10^2 10^3 10^4
FL1-H

Fig.4c.

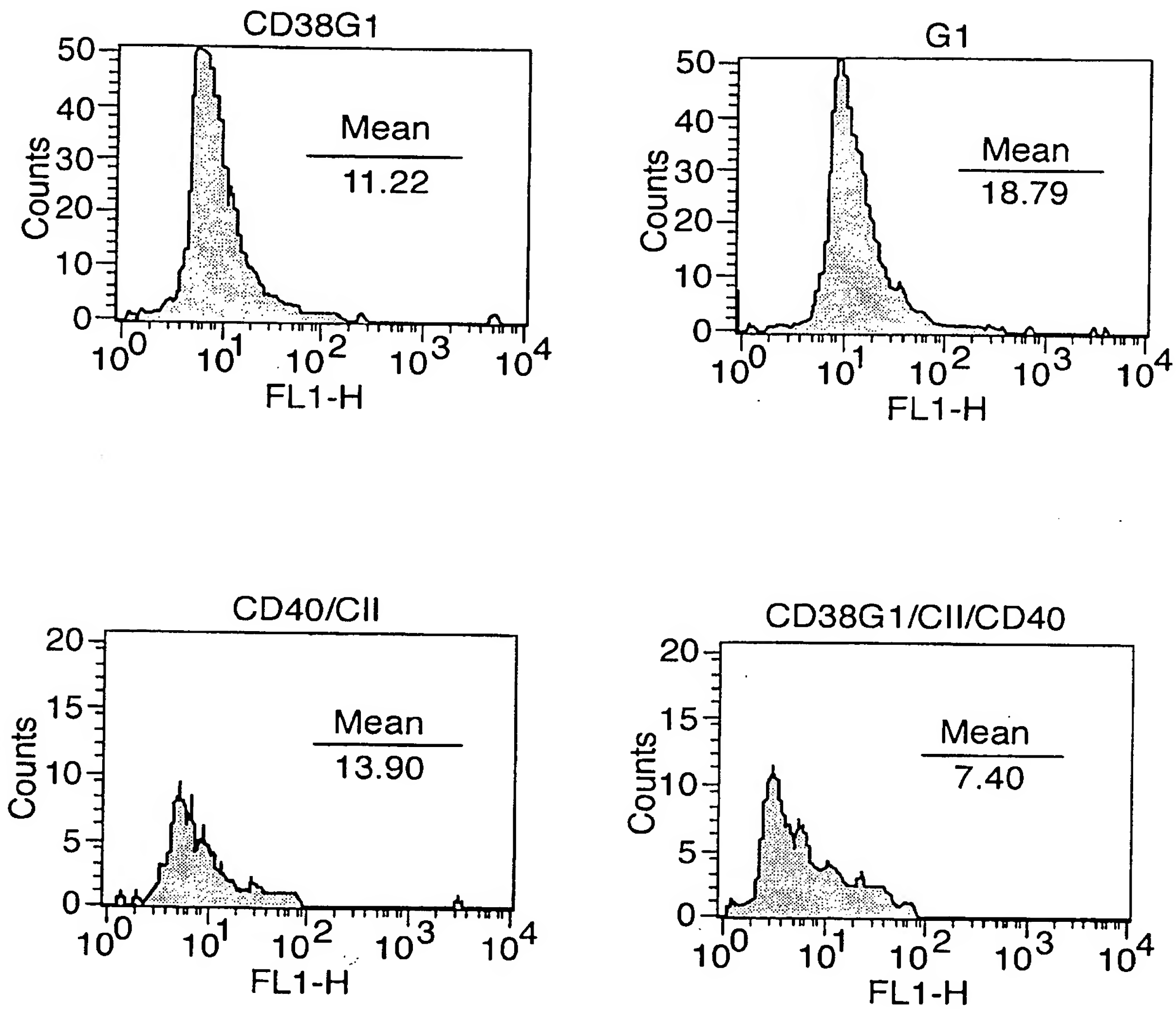

CD38G1
Counts
50
40
30
20
10
0
Mean
11.22
10^0 10^1 10^2 10^3 10^4
FL1-H
G1
Counts
50
40
30
20
10
0
Mean
18.79
10^0 10^1 10^2 10^3 10^4
FL1-H
CD40/CII
Counts
20
15
10
5
0
Mean
13.90
10^0 10^1 10^2 10^3 10^4
FL1-H
CD38G1/CII/CD40
Counts
20
15
10
5
0
Mean
7.40
10^0 10^1 10^2 10^3 10^4
FL1-H

Fig.4c(Cont).

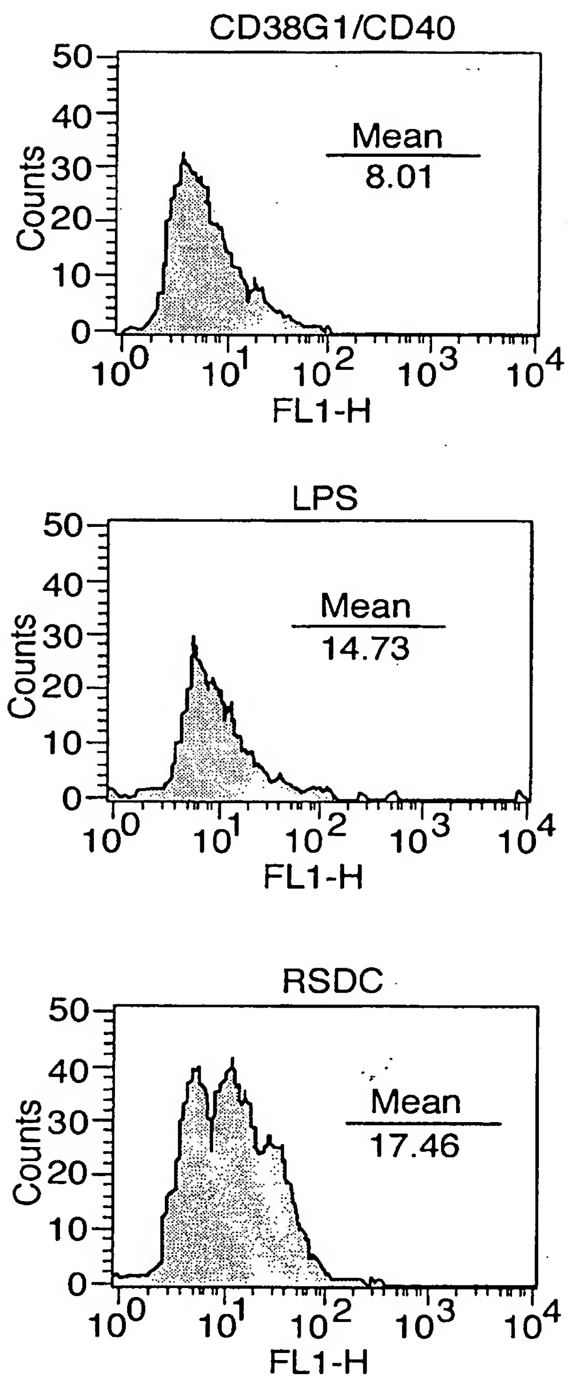

CD38G1/CD40
Counts
FL1-H
Mean
8.01
LPS
Counts
FL1-H
Mean
14.73
RSDC
Counts
FL1-H
Mean
17.46

Fig.4d.

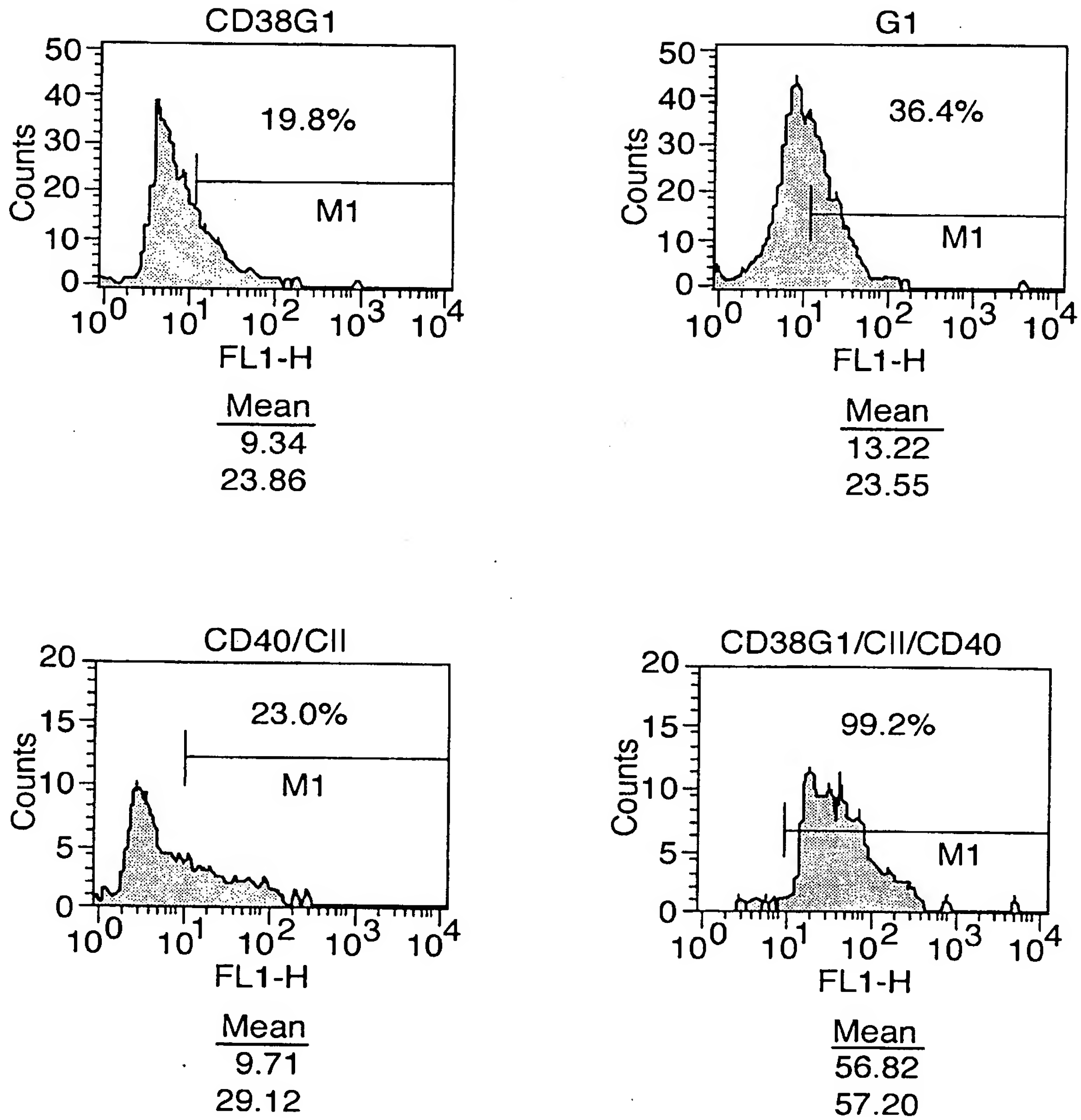

CD38G1
19.8%
M1
Counts
FL1-H
Mean
9.34
23.86
G1
36.4%
M1
Counts
FL1-H
Mean
13.22
23.55
CD40/CII
23.0%
M1
Counts
FL1-H
Mean
9.71
29.12
CD38G1/CII/CD40
99.2%
M1
Counts
FL1-H
Mean
56.82
57.20

Fig.4d(Cont).

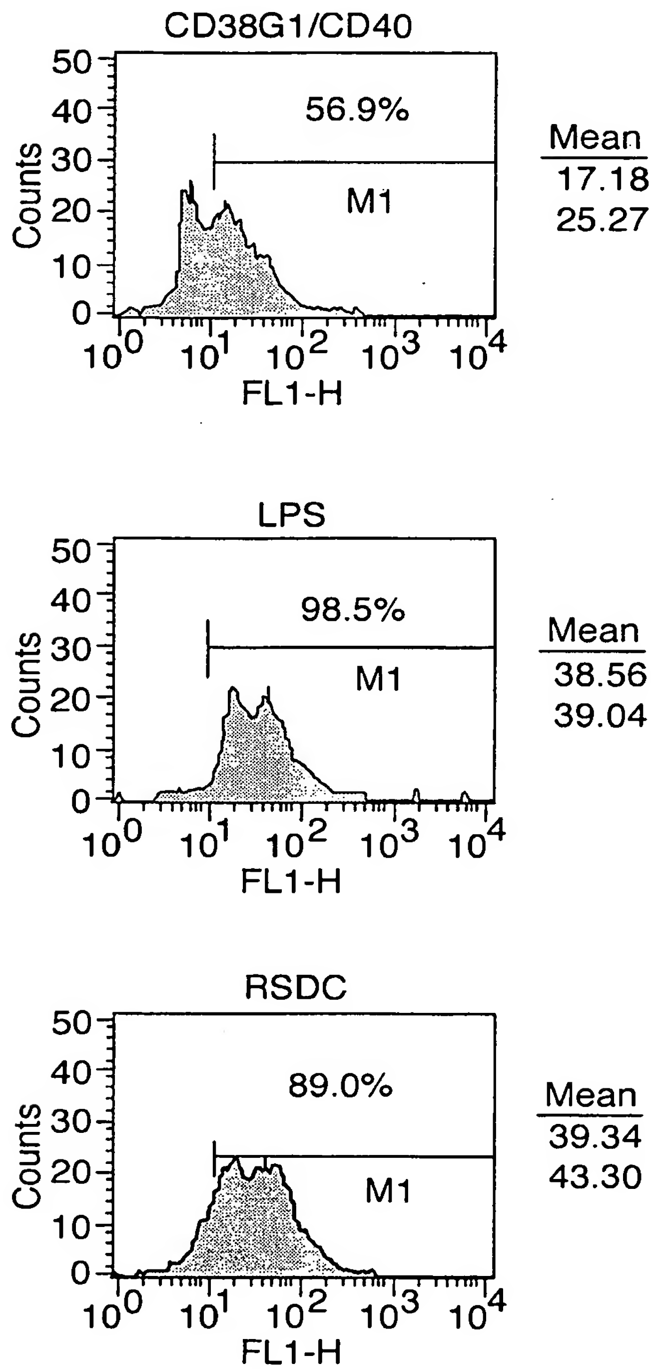

CD38G1/CD40
56.9%
M1
Counts
FL1-H
Mean
17.18
25.27
LPS
98.5%
M1
Counts
FL1-H
Mean
38.56
39.04
RSDC
89.0%
M1
Counts
FL1-H
Mean
39.34
43.30

Fig.5a.

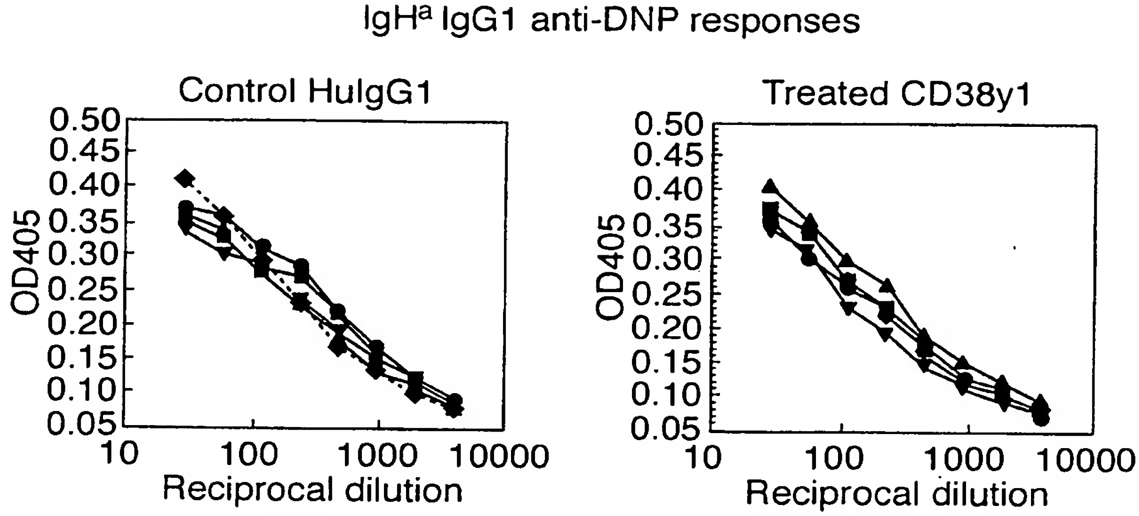

IgHa IgG1 anti-DNP responses
Control HuIgG1
Treated CD38y1
OD405
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
10
100
1000
10000
Reciprocal dilution

Fig.5b.

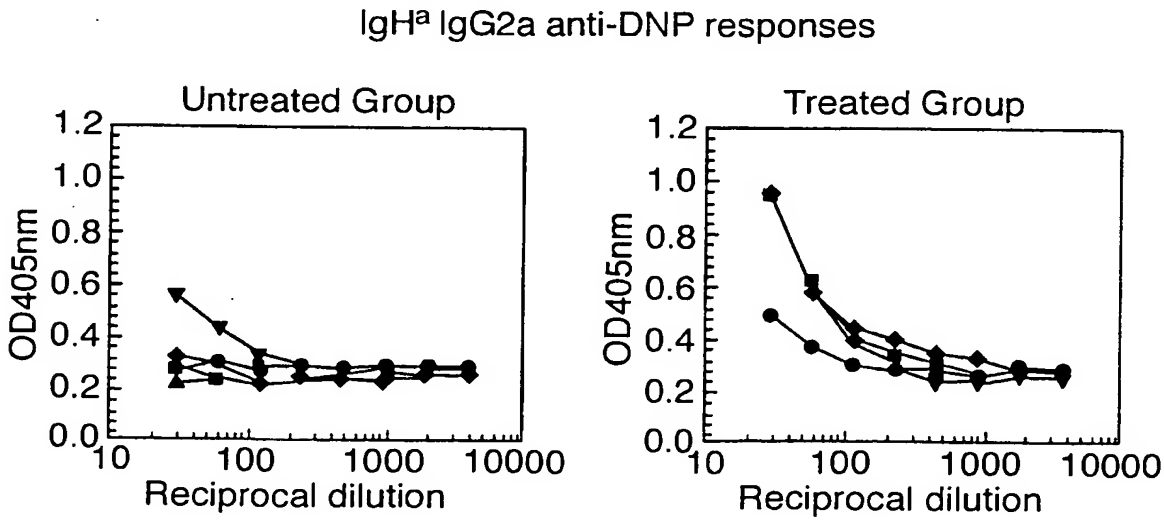
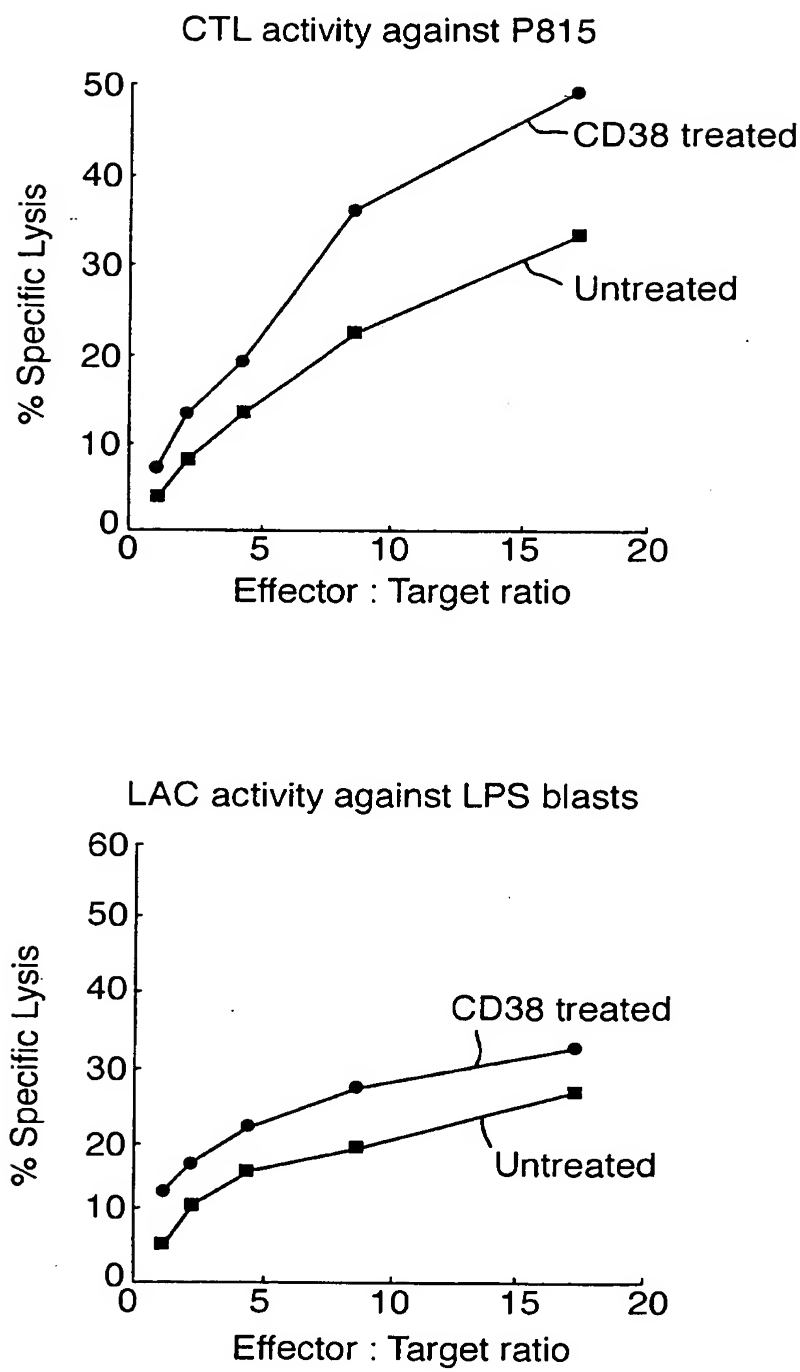

IgHa IgG2a anti-DNP responses
Untreated Group
Treated Group
OD405nm
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
100
1000
10000
Reciprocal dilution

Fig.6.


CTL activity against P815
50
40
30
20
10
0
% Specific Lysis
CD38 treated
Untreated
0
5
10
15
20
Effector : Target ratio
LAC activity against LPS blasts
60
50
40
30
20
10
0
% Specific Lysis
CD38 treated
Untreated
0
5
10
15
20
Effector : Target ratio